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Gametogenesis and fecundation in *Zea Mays* as the basis of xenia
and heredity in the endosperm

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(WITH PLATES 6 AND 7 AND TWO TEXT FIGURES)

In the almost endless number of varieties of maize there is afforded excellent material for experimental work on heredity, as is indicated by the part that the plant has played in the genetic studies of the past twenty years; but, unfortunately, maize offers at the same time limited opportunity for satisfactory cytological work. In addition to its other advantages, maize has a variable endosperm, in the transmission of whose characteristics we get not only a deeper insight into the true nature of the angiosperm endosperm but also some interesting checks on general theories of heredity. But many of the conclusions drawn from these endosperm studies have been based upon the assumption of certain facts concerning gametophyte development and a single description of the fecundation process, of which no figures were given. It was for the purpose of putting the cytological side of the question upon a substantial basis that this study was undertaken. The structure and development of the spikelets have been described in other papers (23, 24), and only the essential details will be repeated.

THE MEGASPORE

Before the integuments have made any considerable progress in enclosing the nucellus, the archesporium becomes visible as a

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large hypodermal cell near the tip of the nucellus (PLATE 6, FIG. 1). It soon divides periclinally, giving rise to a parietal cell, the tapetum, and a megaspore mother-cell; but no wall is formed, and the tapetal cell is immediately consumed (FIG. 2). The parietal layer of tissue, five to eight layers of cells in thickness, which is ultimately present (FIG. 16), is formed from epidermal cells by periclinal divisions (FIG. 2 and TEXT-FIG. 1).

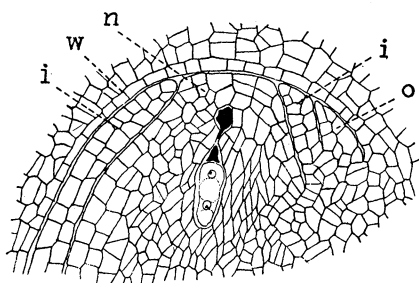


FIG. 1. Binucleate stage of megaspore germination, to show surrounding tissues, $\times 220$: *ii*, inner integument; *o*, outer integument; *w*, ovary wall; *n*, tip of nucellus.

In another connection (24, p. 492) the statement was made that, inasmuch as no evidence had been seen of the disorganization of any of the megaspores, all four probably functioned as in the lilies. But it has since been pointed out to me by Mr. E. G. Anderson, of Cornell University, that, if such be the case, the results of certain experiments on heredity in the en-

dosperm must receive a new interpretation. Consequently, a thorough investigation of the development of the megaspore and embryo sac was thought profitable.

The heterotypic division of the mother-cell is, in all essential details, similar to that in other plants (FIGS. 3, 4). The chromosomes are small and the karyokinetic figures indistinct; and the material is not satisfactory for giving any light on controverted questions of cytology in general. The bivalent character of the chromosomes at this stage is, however, beyond question. A wall is laid down between the two cells resulting from this division (FIG. 5).

The more deeply-seated of these two cells next divides, giving rise to two megaspores (FIG. 6). The other cell begins to divide, but the process is arrested, usually at about the spindle stage, by the incipient disintegration of the dividing cell and the adjacent megaspore (FIG. 7). Thus, only the chalazal one of the four megaspore potentialities persists (FIG. 8). The absorption of the other three is not completed until the embryo sac is almost mature (FIGS. 8-12).

THE EMBRYO SAC

The germination of the megaspore is the same as that in most plants where an ordinary seven-celled embryo sac is to be formed. In the two-nucleate stage (FIG. 9) there appears a large central vacuole, accompanied by a smaller one at the chalazal end of the cell; these vacuoles persist through subsequent stages until the ultimate organization of the embryo sac. The position of the spindles following the four-nucleate stage (FIG. 11) and the arrangement of the following eight nuclei (FIG. 12) substantiates the observation made on numerous other plants that one of the polar nuclei is the sister of the egg.

When the membranes are first formed (FIG. 12), dividing the gametophyte into cells, the antipodals are much larger than the cells of the egg apparatus; but, by the time the embryo sac is fully organized (PLATE 7, FIG. 13), the egg and the synergids have greatly enlarged, and, with little or no increase in size, the antipodal cells have begun to divide, forming the multicellular antipodal tissue which seems to characterize the embryo sacs of most grasses. Meanwhile, the polar nuclei have come together near, or in contact with, the plasma membrane of the egg.

After its organization, the embryo sac continues to increase in size, at the expense of the nucellus, until, at the time of fecundation, it has a volume five to ten times as large as it had at the eight-nucleate stage. This growth is accompanied by rapid changes in other parts of the pistil as well. The nucellus grows rapidly and pushes the integuments out against the ovary wall, which, growing less rapidly, is kept tightly stretched over the turgid ovule. The style, or "silk," increases from a length of one or two centimeters to its full length, which may be forty centimeters or more in some varieties, and develops its numerous stigma hairs.

Guignard (10, p. 44) has given a good description of the embryo sac. It occupies relatively a very small part of the nucellus (TEXT FIG. 2). The cells of the egg apparatus are very large (FIGS. 13, 16). The dense cytoplasm of the pear-shaped synergids shows the longitudinal striations characteristic of these cells in many other plants. The nuclei are located well toward the micropylar ends of the cells and react so feebly to stains as often to be indistinguishable. The egg is larger and more rounded than a

synergid, and its cytoplasm is much less dense. The nucleus is almost centrally located and is closely surrounded by most of the cytoplasm of the cell. It is a little larger than that of a synergid and always stands out distinctly; the nucleolus is large, but the chromatin granules are small and scattered.

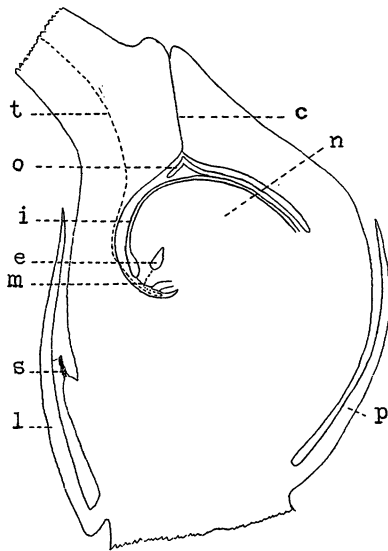


FIG. 2. Longitudinal section of female floret, for purpose of orientation, $\times 75$: *l*, lemma; *p*, palea; *e*, embryo sac; *n*, nucellus; *o*, outer integument; *i*, inner integument; *m*, micropyle; *c*, stylar canal; *t*, course of pollen tube after reaching the ovary; *s*, rudimentary stamen.

The polar nuclei resemble the egg nucleus but are often a little larger. They are usually surrounded by a dense mass of cytoplasm, which often makes detailed observation difficult. They do not fuse until after the entrance of the pollen tube to the embryo sac, even though pollination be artificially delayed until long after the normal time.

The antipodal tissue continues to grow until the time of fecundation, often consisting of fifty or more cells, some of which may have more than one nucleus. A number of these have been observed in the process of division, and, while accurate counts of the chromosomes have not been

made, there is nothing in the appearance of the karyokinetic figures to indicate that there are any more than the haploid number as has been reported for many other plants. The theoretical significance of this antipodal tissue in the grasses has never been fully investigated, and it may help some time to explain the female gametophyte of the angiosperms. It is probably the equivalent of the endosperm of gymnosperms, but in maize it is completely absorbed soon after fecundation and never becomes a part of the endosperm of the mature seed.

THE POLLEN TUBE

As has already been noted (24, p. 487), the generative cell of the pollen grain has divided, forming two very small, crescent-shaped sperms, before the dehiscence of the anther. The pollen grain finally comes to rest on the silk where it is probably held by some sticky exudation that may be instrumental in initiating germination. The pollen tube emerges very soon and makes its way down through one of the stigma hairs to the body of the silk (FIG. 14). Here it pushes its way between the parenchyma cells, absorbing food from them, no doubt, until it reaches one of the vascular bundles, which it follows toward the ovary.

At the time of its emergence from the pollen grain, the tube is greatly exposed to desiccation, and this continues until it has made its way into the body of the silk. This, together with the fact that the pollen grains themselves dry up very readily and are seldom viable for more than a day or two, probably accounts for the importance to the corn crop of warm rains at the time of pollination.

In some kinds of corn the silks may attain a length of fifty centimeters or more, and there are probably few species of plants in which the pollen tube has farther to grow. Consequently, it has been an interesting problem to determine the time elapsing between pollination and fecundation. A number of experiments were made to determine this, and the best results were obtained with a small variety of sweet corn with silks about twenty-five centimeters in length. Silks that had been properly protected were pollenized at 2:45 P.M. on a warm, foggy day in early September, with pollen that had been shed on the forenoon of the same day, and ovules and segments of silks were fixed at various intervals for two or three days after pollination. It was found that within two hours the pollen tubes were well established in the silks. This result has also been verified by several experiments in the laboratory (FIG. 14). Ovules fixed twenty-five hours after pollination showed fecundation and stages just before (FIG. 16) and just after fecundation. These results could, of course, be modified by extensive experiments with silks of different lengths and pollen of different ages and at different temperatures, degrees of humidity, etc., but that is rather the problem of the physiologist.

The aim in presenting here the meager data available is to show the great rapidity with which the pollen tube grows. It is interesting to note that Jensen (13, p. 15) finds a somewhat greater time (32 to 40 hours) between pollination and fecundation in wheat, although the style of the latter is much shorter than in maize.

It is not to be inferred that there is at any one time a living pollen tube reaching from the stigma hair to the embryo sac; and this statement is probably very generally true of all angiosperms. Observation of the pollen tube in the silk is difficult, but it is probable that there is not more than a centimeter or two of it alive at any one time; and the living portion seems to become shorter and broader as it approaches the ovule. It is in most cases practically impossible to find any trace of the pollen tube in the tissue through which it has passed.

One of the difficulties in attempting to observe fecundation is in being able to identify the sperms when they reach the embryo sac. The protoplasm of the pollen tube, as it grows down through the silk, is so dense and so coarsely granular that it is almost impossible to distinguish with certainty the sperms and the tube nucleus. Both can be made out with a fair degree of certainty, however, in the less dense contents of the tube in the ovary cavity as it approaches the embryo sac; and they are, of course, visible in the pollen grain before germination. The tube nucleus is irregular in size and shape and does not seem to have a definite membrane. There is no visible difference between the two sperms. They are very small, slender crescents, with the ends drawn out to very fine points (FIGS. 15, 16). The nucleus, which seems to consist almost wholly of chromatin, constitutes the greater part of the cell and forms the middle of the crescent; no nucleolus has been observed. There is only a small quantity of cytoplasm, and nothing of its details can be made out.

The size of the sperm is remarkably correlated with the long distance through which it must be carried from the pollen grain to the embryo sac. Practically all varieties of maize are fertile *inter se*; and this means that any pollen grain, even though it be produced by a plant characterized by short silks, is capable of producing a pollen tube as long as is necessary, as is determined by the length of the silk of the variety pollenized.

The path of the pollen tube on reaching the ovary has never been satisfactorily explained. True (20, p. 217) quotes Hackel (11) as saying that the outer integument acts as a conducting tissue. Guignard (10, p. 43) says that the tube enters the ovary by way of the stylar canal. My preparations have not given conclusive results, but they do not tend to substantiate these opinions. The pollen tube seems to grow downward from the base of the style until it reaches the inner epidermis of the wall of the ovary (TEXT FIG. 2). Remnants of it can be traced just below the epidermis to a point near the micropyle, where it enters the ovarian cavity. After following a very crooked course here, it finally enters the micropyle, forces its way between the cells of the parietal tissue, and enters the embryo sac (FIG. 16).

FECUNDATION

The contents of the pollen tube may be emptied between the cells of the egg apparatus or into any one of them; but it is usually emptied into a synergid. The membrane of the other synergid often seems to break at about the same time, emptying its contents into the cavity of the endosperm cell. The tube nucleus is soon lost to view, if it was visible at all; and, because of their extremely diminutive size, it is very difficult to follow the sperms beyond this point. The cytoplasm of the area to be examined is very dense and is filled with numerous globules of various sizes and shapes (FIGS. 13, 16), produced, no doubt, by the mixing of the cell contents brought together by the entrance of the pollen tube. The disorganizing synergids give rise to cytoplasmic differentiations that are especially confusing. Material fixed with reagents containing osmic acid presents almost hopeless complications; but, with chromo-acetic acid as a fixing agent, followed by Flemming's triple stain, the sperm nuclei can be identified.

In several preparations a small red object, which is like a sperm nucleus in size, shape, and granular appearance, has been seen sticking to the egg nucleus (FIG. 17); and a similar body has, in a number of instances, been seen attached to one (FIG. 18) or both (FIG. 19) of the polar nuclei, which were in the process of fusion. No fusing polar nuclei have been seen without this sperm-like body. On this basis, then, it seems certain that the so-called "double fecundation" takes place in maize.

In 1901 Guignard (10) reported having observed this process, but his otherwise excellent paper was not illustrated. While his results have been generally accepted by morphologists, and made the basis of extensive work by geneticists, the desirability of a verification is attested by the number of students who are known to have attempted at different times to repeat his work. If negative results were generally published, we should, no doubt, have a much more voluminous literature on this point.

In one preparation the writer observed the two fusions taking place simultaneously (FIGS. 17, 18), but in a majority of cases one preceded the other. Whether or not there is a definite order in which the fusions occur has not been determined, because it is almost impossible to determine by appearance alone whether a cell under observation is an egg or a zygote. It is true in all cases, however, that several free endosperm nuclei are formed before the first division of the fecundated egg.

At the time of fecundation the egg nucleus is often seen to have moved to one side of the cell (FIG. 16), and the polar nuclei may also migrate within a limited range, often approaching the micropylar end of the embryo sac. Whether or not there is any significance to be attached to these migrations has not been determined.

These fusions take place while the egg nucleus and the polar nuclei are in a resting condition (FIGS. 17-19). The chromatin is gathered into numerous round, globular bodies, some of them almost as large as a sperm; and these granules are loosely connected by strands composed of finer granules of similar appearance. That these chromatin bodies are not the "prochromosomes" of some authorities is attested by their number, which is far too great.

XENIA

As long as maize has been cultivated, it has been noted that, when white and colored varieties of some kinds were grown close together, the ears of the former were likely to bear a few colored seeds. The American Indians are said to have observed this and to have attributed it to the intermingling of the roots underground. Later, civilized man attributed the phenomenon to some effect of cross pollination, but its mechanism long remained a mystery.

In 1881, Focke (9, p. 511) coined the word *xenia* to apply to the immediate effect of foreign pollen upon maternal tissue.

Nawaschin's discovery (16) that in many plants, one of the sperm nuclei enters into the makeup of the endosperm, suggested an explanation of the mechanism of *xenia* in maize; and Guignard's discovery of "double fecundation" in maize, in 1901 (10), left no room for reasonable doubt as to the mechanism of *xenia*.

The immediate effects of cross pollination, that is, such effects as may immediately be observed in the seed or fruit in which the hybrid embryo is borne, are probably not so common as has often been supposed; and by no means are all of them illustrations of *xenia*, in the true sense of the word.

There is a common belief that pumpkins growing near watermelons will, by hybridization, impair the quality of the latter in the first generation; and numerous other instances have been cited of slight changes in the quality of fruits, supposed to be due to the immediate effects of cross pollination. The experimental demonstration of most of these, with races known to be genetically pure for the characteristics considered, has not been accomplished.

Another type of the phenomenon is afforded by hybrids between varieties of peas and other plants, which are variable as to the color or physical character of the cotyledons. Here a dominant character may, through hybridization, appear in seeds borne on plants pure for the recessive character. But this is merely the early recognition of a hybrid by means of characteristics that are differentiated in the embryo. Bailey and Gilbert (1) must have had in mind such phenomena as these when they made the erroneous statement (p. 327) that *xenia* occurred in peas.

In contrast with these phenomena is the immediate effect upon the endosperm, so well known in maize. This has also been demonstrated in teosinte-maize hybrids (21), and has been reported in a few other crosses between different varieties of cereals. The primary essential for *xenia* is *variability of endosperm* in plants that will hybridize, and for this reason, maize when used for one of the parents, at least, furnishes the best-known illustration.

In a recent review of the whole question of influences following fecundation, Waller (22) suggests that the term *xenia* be reserved for the phenomenon limited to the endosperm of angiosperms and

due to the entrance of a sperm into the constitution of the primary endosperm nucleus. It is a direct result of the introduction of hereditary factors into the endosperm and not to be attributed to enzymatic action or other stimulating influence. To include "those influences which follow fertilization but are remote from it" (p. 282) and are "due to the developing zygote" (p. 284), he proposes the term *ectogony*.

Whether or not the new term is applicable, is not a question to be decided in this connection; but the distinction between xenia and other influences less directly connected with fecundation is a timely one. Xenia may be defined, then, as *any effect that may be produced upon the endosperm of an angiosperm by pollination with pollen from a plant having a different kind of endosperm*.

Of course, it is not to be expected that xenia will occur in all such crosses. Correns (3, pp. 411-414) has outlined a number of cases in which xenia will or will not occur in maize, and East and Hayes (7, p. 103) have condensed all the available data into a law of xenia. For our purpose, it may be said that xenia will occur in any cross in which the male parent possesses the dominant and the female the recessive of an allelomorphic pair of endosperm characters, or when the two parents possess respectively two characters whose interaction is necessary for the production of a visible effect. When dominance is incomplete, or when inhibiting factors are present, complications are introduced which need not be discussed here.

THE ENDOSPERM OF ANGIOSPERMS

The variability of the maize endosperm, with the accompanying phenomenon of xenia, gives it a prominent place in any explanation of the endosperm of angiosperms. This tissue has been explained in many ways, but its true significance still offers an unsolved problem. The correct explanation, if it is ever found, will probably develop from researches on the phylogenetic origin of the angiosperms.

In most of the plants in which fecundation has been studied, one sperm has been found to enter into the constitution of the endosperm, and the phenomenon is believed to be of general occurrence. But there are many exceptions which serve to com-

plicate the problem. A good résumé if these is given by Coulter and Chamberlain (5, pp. 165-186), and more are being added from time to time.

Miss Sargant (17, p. 702) stated clearly the problems involved in the interpretation of the triple fusion and reviewed the explanations that had been given up to 1900. Although her paper appeared very early in the investigation of the question from the modern point of view, it showed such a clear insight into the nature of the problem that the data that have accumulated since that time have afforded little foundation for further constructive work.

Some have considered the endosperm merely a belated prothallium, the triple fusion of nuclei being non-sexual and of no more significance than the nuclear fusions that often occur in vegetative tissue. Strasburger (18, p. 308) calls the triple fusion a "vegetative fertilization," as contrasted with the "generative fertilization" of the egg. These types of fecundation resemble each other in the transmission of certain hereditary characters, but they differ in the nature of the new individuals produced.

Again (17, pp. 704-706), the endosperm may be looked upon as a monstrous embryo, its aberrant nature and limited development being determined by the antipodal one of the polar nuclei, inasmuch as its chromosome number is known to be irregular in many plants. According to this view, the endosperm is neither sporophyte nor gametophyte, but a new generation, characteristic of the angiosperms alone, and for which Trelease (19) has proposed the name *xeniophyte*, because it is the generation in which xenia may occur.

All agree upon the function of the endosperm as an organ of nourishment for the embryo, and its rapid growth has been attributed to its sexual, or pseudo-sexual, origin. Collins and Kempton (2) and Jones (14) have even shown that hybrid endosperms, produced by crossing varieties of maize that have been selfed for some time, show a perceptible increase in size over those of the parent races. This is attributed to the increased vigor usually resulting from hybridization. Miss Sargant (17, pp. 709-710) has suggested that the introduction of the paternal elements into the primary endosperm nucleus may be a device of the plant to pro-

duce a food of proper quality for the hybrid embryo. In the light of later work on xenia, we should be compelled to infer from this that the peculiar endosperm qualities necessary in such cases were the *dominant* characters, since we have no evidence that, except in special cases to be mentioned later, the recessive characters of the male are ever effective in the endosperm; they are not visible, and there is no future generation in which they might be detected. Moreover, in a cross between sweet and starchy varieties, the former being the female, there is produced a starchy endosperm, which is harder to digest than the sweet one that would have been produced had the triple fusion not occurred.

The part played by the maize endosperm, then, is to complicate the problem and at the same time act as a check on our solutions. Were it not for xenia and the attendant hereditary phenomena, the logical disposal of the endosperm would be to call it gametophytic tissue resulting from a triple vegetative fusion to which no great significance could be attached; and this would probably have been done long ago had the peculiarities of the maize endosperm not been known. But the transmission of hereditary characters to this tissue, as illustrated in maize and a few other species, strongly suggests its parallelism with the sporophyte and saves us from an incorrect explanation of the ques ion.

HEREDITY OF ENDOSPERM CHARACTERS

Without committing ourselves as to the most logical interpretation of the endosperm in general, we may, for the purpose of explaining genetic data, adopt the convenient expedient of considering the endosperm of maize a monstrous sporophyte, a sort of sister of the embryo. It derives a vigorous growth stimulus from the triple fusion in which it originates. It passes through a series of tissue differentiations, none of which, however, resemble very closely those characteristic of the embryo. It never reaches sexual maturity, and, consequently, has no descendants. Because of the sporophytic nature of the endosperm, Mendelian principles of heredity have been applied to its study in maize, with results unique in many instances, and all dependent, more or less, upon the cytological facts here set forth, or, at any rate, nor at variance with them.

The maize endosperm is either sweet or starchy. The starchy tissue occurs in two forms, one corneous and translucent, and the other soft and white; and different proportions and variations in the arrangement of the soft and corneous portions gives rise to dent, flint, pop, and soft types. The mature seeds of the sweet varieties are always wrinkled; they are to be understood as having pop, dent, flint, and soft potentialities remaining invisible because of limited starch development. The starchy condition is dominant to the non-starchy, but dominance among the variations of the starchy condition is a less definite thing.

The corneous endosperm is either white or yellow; the yellow color, which has been found to be due to more than one hereditary factor, is dominant. It may appear in either the starchy or the sweet endosperm.

The aleurone is red, purple, or colorless. Two hereditary factors are necessary for the production of the red color, and these, interacting with a third, produce purple. The presence of either color combination is dominant to its absence, but other color genes are also present, at least one of which is an inhibiting factor.

Correns found (4) in certain crosses that a white variety pollenized with pollen from a purple did not always produce purple aleurone, although the embryos of the same seeds proved to be hybrids. He explained this non-appearance of xenia by assuming that the recessive factors carried by the two maternal nuclei entering into the primary endosperm nucleus were dominant over the one factor introduced by the sperm, although the latter was ordinarily dominant.

East and Hayes (7, pp. 58-59), having found a better explanation of this aberrant result, attacked Correns's hypothesis on the ground that quality and not quantity of chromatin is the determining factor. In this argument they failed to distinguish between the idea of double quantity and quality acting twice.

In later experiments (12, p. 12) they found that when reciprocal crosses were made between a soft and a corneous variety, the quality of the endosperm produced was always determined by the female parent. At the same time it was shown that a soft white female crossed with a flinty yellow male produced soft yellow seeds. The appearance of xenia in color showed that the peculiar

results of the reciprocal crosses were not due to a failure of the sperm to fuse with the polar nuclei, as Webber had suggested (25, pp. 34-37) in explanation of a similar occurrence. Therefore, they were forced to conclude that two applications of the one factor may dominate one application of the other.

Results leading to the same conclusions have since been secured with color combinations. If a plant heterozygous for purple aleurone be selfed, four types of endosperm should be produced, depending upon the number of times the factor combination for purple is present. And when such an experiment is made, there are produced, besides the proper number of white seeds, visibly different types of purples, appearing in significant ratios. Emerson (8) has recently given a detailed report on a number of experiments of this kind.

It may be said, therefore, that the solution proposed by Correns was a valid one, although it did not apply to the problem that he had in hand. By taking advantage of the triple parentage of the endosperm, this series of experiments provided the first direct evidence of the cumulative effect of repeated applications of the same factor, which is the basis of the multiple factor hypothesis.

But these theoretical conclusions have all been based upon the assumption of certain cytological facts not hitherto demonstrated. If, in the formation of the maize embryo sac, all four megaspores functioned, which the writer (24, p. 492) at one time thought probable, the two polar nuclei, coming respectively from the two nuclei resulting from the heterotypic division, would be genetically different in a hybrid plant. Therefore, a sweet-starchy hybrid, crossed with pure sweet, should produce all starchy seeds, since the two polar nuclei together would represent an entity unaffected by segregation and always carrying the dominant factor. But, as a matter of fact, when a cross of this kind is made, a 1 : 1 ratio is produced. The assumption that only one megaspore survived had good precedent in Koernicke's work (15) on *Triticum*, recently verified by Jensen (13); but now it is definitely known that the same is true of maize.

In some crosses of white varieties with purple or red, a mottled aleurone is produced; and in sweet-starchy crosses a few seeds have been found with the endosperm bilaterally divided—one

half starchy and one half sweet. Webber (25, pp. 34-37) found an explanation for such irregularities in the assumption that the sperm might sometimes fail to fuse with the polar nuclei, and thus be left to divide independently, or might fuse with only one of them, the other dividing independently. In either case there would be two, possibly genetically different, sources of endosperm formation, which might account for the two colors of aleurone. It has since been found that aleurone mosaics are amenable to Mendelian principles, but for the divided endosperms East and Hayes (7, pp. 34-35) consider Correns's explanation well-founded.

This assumption requires that either the sperm or one of the polar nuclei, both of which have been considered gametic in nature, develop without uniting with another gamete, which would be a very unusual behavior. If such an explanation of the occurrence is necessary, it seems more reasonable to suppose that the sperm united with the polar nucleus coming from the micropylar end of the embryo sac, since it is the sister of the egg and necessarily much like a gamete, and that the antipodal one of the polar nuclei, with its possibly irregular chromatin organization, divided independently, as its near relatives, the true antipodals, regularly do in maize and other grasses. Yet this would take from one polar nucleus much of its gamete-like nature and might seriously interfere with its ability to transmit hereditary characters in the orthodox fashion necessary for some of the other phases of our theory. In fact, any assumption of this kind will lead to the conclusion that something, elsewhere considered gametophyte, here contributes directly to endosperm formation and shows endosperm (xeniophyte) characteristics.

The responsibility for these rare occurrences of bilaterally differentiated seeds may be placed upon still another hypothesis which involves valid cytology and is not in conflict with Mendelism. The division of the primary endosperm nucleus may be heterotypic in a way. In fact, it is well known that the subsequent divisions often show fewer than $3x$ chromosomes; some chromosomes must, therefore, at some time, pass bodily to the poles of the spindle without themselves dividing, and, if one of these carries the gene for starchiness, reduction necessitates a sort of Mendelian segregation.

In the frequency with which these bilaterally differentiated endosperms occur—about one in 10,000—East (6, p. 220) has likened them to “bud sports.” This explanation is not materially different from the one suggested above, although the cytological phenomena accompanying the formation of “bud sports” are not well understood at present. In the endosperm, where chromatin behavior seems much less regular than in ordinary embryonic tissue, the chances for such occurrence seem exceptionally good; but, neither in the division of the primary endosperm nucleus nor in the arrangement of the free nuclei in subsequent stages, is there anything to account for the perfect symmetry and fine line of demarcation characterizing these seeds.

SUMMARY

Of the four potential megaspores in maize, only one persists and becomes functional.

The embryo sac is a modified form of the ordinary seven-celled type. The antipodals undergo division, forming a large mass of tissue before the time of fecundation.

The sperms, which are exceedingly small, are matured inside the pollen grain before the dehiscence of the anther.

External conditions and the length of the silk determine the time required for the growth of the pollen tube, but fecundation is known to have taken place in some instances within a little more than twenty-four hours after pollination.

Guignard's work on double fecundation in maize is verified. Both sperms are functional, one uniting with the egg to give rise to the embryo, and the other entering into the constitution of the primary endosperm nucleus. This is the cytological basis of xenia and the attendant hereditary phenomena. Immediate effects of pollination made manifest outside the embryo, if such really occur, should not be called xenia.

The triple fusion takes place almost simultaneously with fecundation of the egg, but the endosperm develops much more rapidly than the embryo.

In connection with genetic studies, the assumption that the endosperm is sporophytic in its genetic behavior seems consistent. The cytological data substantiate practically all the assumptions

of cytological fact upon which recent experiments on endosperm heredity in maize have been based.

I take opportunity here to express my obligations to Professor D. M. Mottier for the suggestion of this problem and for valuable assistance and criticism in connection with the study.

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Explanation of plates 6 and 7

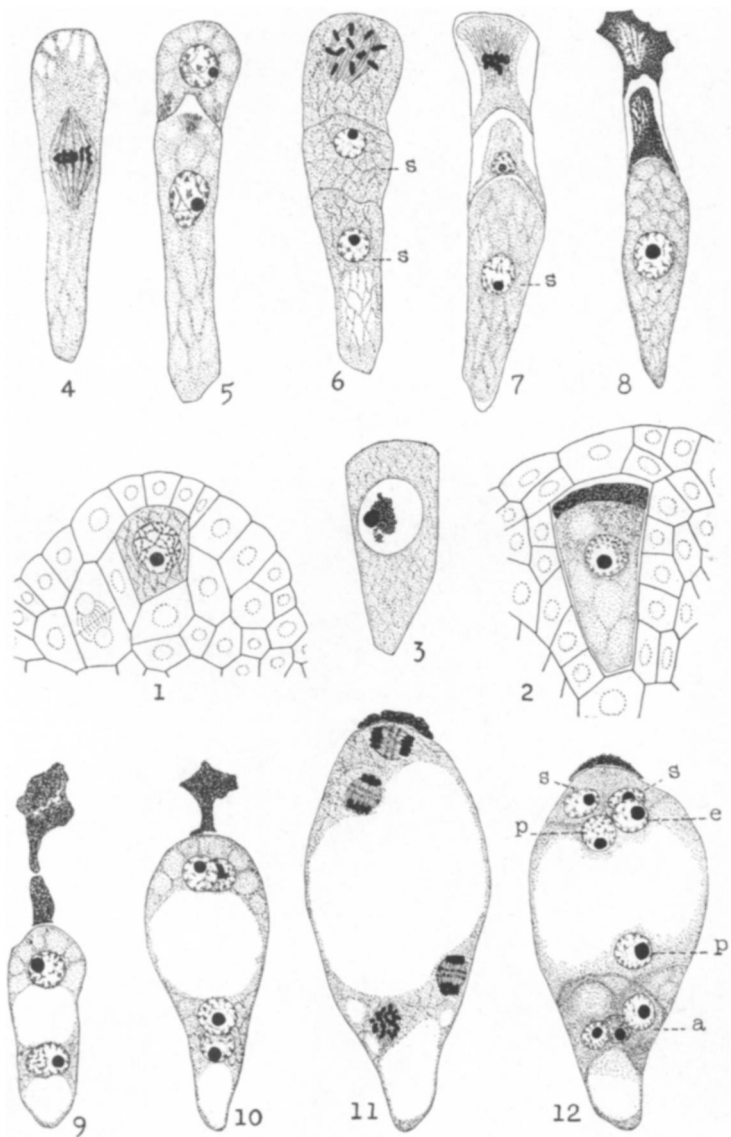
PLATE 6

All figures $\times 725$

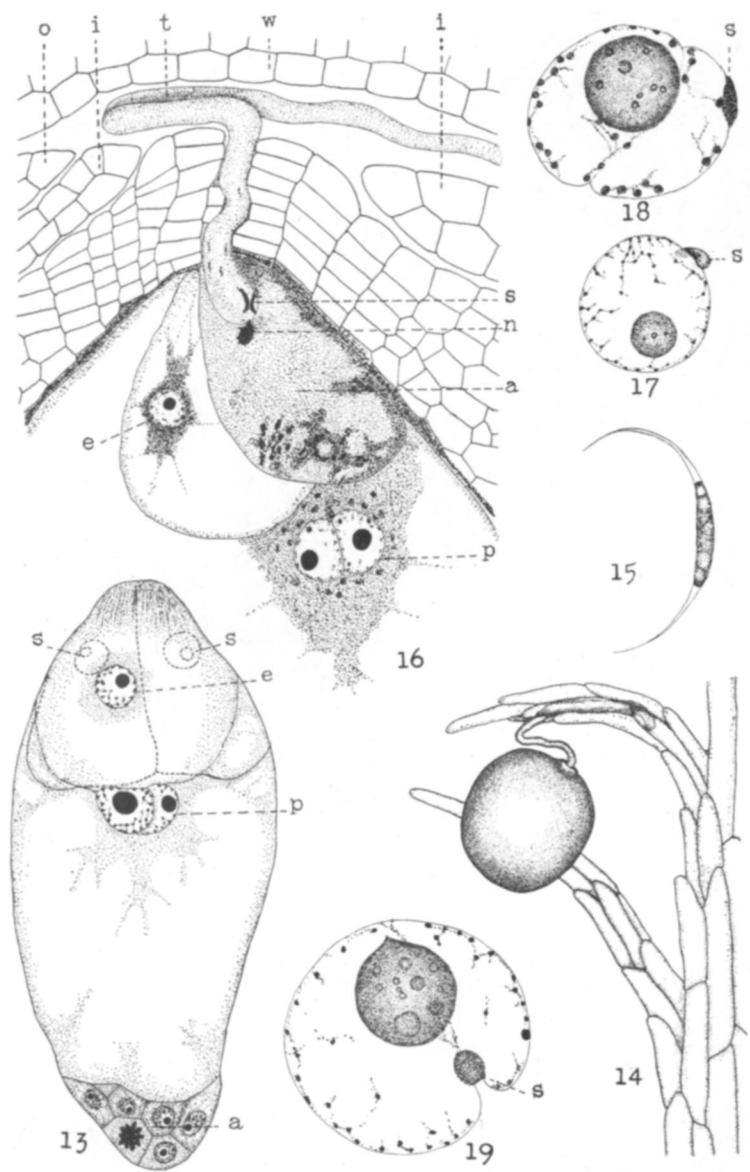
- FIG. 1. The archesporial cell.
 FIG. 2. Megaspore mother-cell and disorganized tapetal cell.
 FIG. 3. Megaspore mother-cell in synapsis.
 FIG. 4. Heterotypic spindle.
 FIG. 5. Daughter cells resulting from reduction division.
 FIG. 6. Two megaspores, *ss*, and micropylar daughter cell in process of division.
 FIG. 7. Dividing daughter cell and one megaspore beginning to disintegrate: *s*, functional megaspore.
 FIG. 8. Functional megaspore absorbing the other three.
 FIGS. 9-12. Steps in the germination of the megaspore and organization of the embryo sac: *ss*, synergids; *e*, egg nucleus; *pp*, polar nuclei; *a*, antipodal cells.

PLATE 7

- FIG. 13. Embryo sac soon after organization, $\times 725$: *ss*, synergids (position shown by dotted lines); *e*, egg nucleus; *p*, polar nuclei; *a*, antipodal tissue.
 FIG. 14. Germinating pollen grain on stigma hairs, $\times 200$.
 FIG. 15. Sperm, $\times 1750$.
 FIG. 16. Pollen tube entering embryo sac, $\times 385$: *o*, outer integument; *ii*, inner integument; *t*, pollen tube; *w*, ovary wall; *s*, sperms; *n*, tube nucleus (probably); *e*, egg nucleus; *p*, polar nuclei in contact; *a*, one synergid; other synergid not shown.
 FIG. 17. Sperm nucleus, *s*, in contact with egg nucleus, $\times 1500$.
 FIGS. 18, 19. Sperm nuclei, *ss*, in contact with the fusing polar nuclei, $\times 1500$.



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